Nucleolar Organizer Regions in Patients with Precancerous and Cancerous Lesions of the Uterine Cervix

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ABSTRACT: *Nucleolar organizer regions (Ag-NORs) were studied in patients with cervical precancerous and cancerous lesions along with controls. The data revealed a statistically significant decrease of Ag-NORs in cancer patients, as well as in women with severe precancerous lesions as compared with controls. A similar decrease in the amount of Ag-staining was also observed in both cancer and severe dysplasia cases. The study suggests a possible relationship of Ag-NOR activity to malignancy.*

INTRODUCTION

Nucleolar organizer regions (NOR) in humans are visualized as secondary constriction regions of acrocentric chromosomes of D and G groups by silver staining methods [1, 2]. Hybridization techniques in situ demonstrated that NORs contain the RNA genes for 18s and 28s [3, 4], and somatic cell hybridization techniques further demonstrated that the active NORs are preferentially stained by the silver staining method [5, 6]. The frequency of Ag-NOR was found to be fairly constant in a particular tissue of an individual, although variations also have been reported in different tissues [7–10]. Reports on NOR activity in malignant diseases are contradictory. Some malignant diseases, such as lymphoma, leukemia, and solid tumors, showed fewer or similar number of Ag-NORs to those of healthy individuals [11–13]. On the other hand, an increased frequency of Ag-NORs was reported in the leukocytes of female patients with adenocarcinoma [14] and in hypodiploid tumor cells of meningiomas [15]. In the present communication we report the frequency of Ag-NORs in the leukocytes of female patients with cervical precancerous and cancerous lesions.

MATERIALS AND METHODS

Fifty-three women with cervical precancerous lesions (dysplasias), 16 cervical cancer patients, and 37 healthy women were included in the study. The ages of the three groups were more or less matched. The ages for the controls, precancerous,

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and cancerous groups were 23–65, 22–62, and 22–60 years, respectively. Patients were diagnosed cytomorphologically according to WHO criteria [16].

Of the 53 cases of precancerous lesions, 23, 22, and 8 were from mild, moderate, and severe dysplasias, respectively. Cancer patients and some of the patients having precancerous lesions were diagnosed both cytologically and histopathologically. Whole blood was cultured in TC 199 medium supplemented with 20% heat inactivated human AB serum and phytohemagglutinin-P for 72 hours. Colcemid (0.02 μg/ml) was added 2 hours prior to harvesting. Air-dried chromosome preparations were made by a conventional method. Ag-NOR staining was performed according to Howell and Black's method [17]. The amount of Ag-stain on each acrocentric chromosome was recorded visually as 0 (absent), 1 (small), 2 (medium), or 3 (large), according to Miller et al. [18]. Ten metaphases from each subject were scored for positive silver staining of NORs.

RESULTS

Individual variations in modal numbers of Ag-NORs were observed in the precancerous and cancerous group, as well as in controls (Table 1). The modal numbers varied from 5 to 10 (controls) and 4 to 10 (precancerous lesions) whereas, in cancer patients, the modal number was between 5 and 8.5. The variation of Ag-NORs in cancer cases was lower, and compared with that of normal and precancerous lesion subjects. The average modal number of Ag-NORs was found to be less in cancer cases (6.38), compared with that of controls (7.00). The average Ag-NOR per metaphase was found to be less (6.25 ± 0.88) in the cancer group compared with controls (7.03 ± 0.88). The difference was found to be statistically significant ($p < 0.02$). The modal number of Ag-NORs in cases with precancerous lesions was 7.0, 7.3, and 6.1 in mild, moderate, and severe dysplasias, respectively. Although there was an apparent decrease in the frequency of Ag-NORs in severe dysplastic cases (6.23 ± 1.09), the difference was not significant ($p > 0.05$), compared with those of cases with mild (7.13 ± 1.21) and moderate (7.23 ± 1.18) dysplasias. Ag-NORs in the precancerous group, as a whole, did not differ from that of controls.

The distribution of Ag-NORs was further analyzed to look into variations with respect to D and G group chromosomes. Analysis revealed that the decreased frequency of Ag-NORs in cancer cases was not due to any specific group of chromosomes (D and G), but was evident in both groups (Table 1). The distribution of the modal number of Ag-NORs among the groups is shown in Table 2. The majority of cases of severe dysplasia (87.50%) and cancer (93.75%) revealed modal numbers 7 or less, compared with normal women (56.75%) and other lower grades of dyspla-

Table 1 Frequency of Ag-NORs in different groups of women

Group	Number studied	Silver stained NORs			
		Modal number	Mean ± SD/metaphase	D group	G group
Controls	37	7.0 (5–10)	7.03 ± 0.88	4.16 ± 0.76	2.87 ± 0.70
Dysplasias					
Mild	23	7.0 (4–10)	7.13 ± 1.21	4.39 ± 0.74	2.65 ± 0.78
Moderate	22	7.3 (5–10)	7.23 ± 1.18	4.38 ± 0.91	2.85 ± 0.86
Severe	8	6.1 (4–8)	6.23 ± 1.10	3.90 ± 1.09	2.33 ± 0.49[b]
Cancer	16	6.3 (5–8.5)	6.25 ± 0.88[a]	3.77 ± 0.78	2.48 ± 0.63

[a]Significant in t test: $p < 0.02$.

[b]Significant in t test: $p > 0.05$.

Table 2 Distribution of Ag-NORs in different groups of women

Group	Number studied	Modal number of Ag-NORs				
		6	7	8	9	10
Controls	37	9 (24.32)[a]	12 (32.43)	13 (35.13)	1 (2.70)	2 (5.40)
Dysplasia						
Mild	23	5 (21.74)	8 (34.78)	7 (30.43)	2 (8.69)	1 (4.35)
Moderate	22	5 (22.73)	7 (31.82)	7 (31.82)	1 (4.55)	2 (9.09)
Severe	8	5 (62.50)	2 (25.00)	1 (12.50)	—	—
Cancer	16	9 (56.25)	6 (37.50)	—	1 (6.25)	—

[a]Numbers in parenthesis indicate percentage.

Table 3 Amount of Ag stain per acrocentric chromosome in different groups of individuals

Group	Number of individuals	Ag stain per chromosome		Ag stain per acrocentric chromosome (D+G)
		D group	G group	
Controls	37	1.0388	1.1308	1.0555
Dysplasia				
Mild	23	1.3061	1.1578	1.2468
Moderate	22	1.0089	0.9178	0.9725
Severe	8	0.8816	0.7895	0.8447
Cancer	16	0.8570	0.8602	0.8583

sias (Table 2). Rarely, an individual with cancer had modal numbers 8 or more (6.25%), compared with controls (40.74%). Further, none of the groups showed any age relationship with Ag-NORs by regression analysis.

The results on the amount of Ag stain per acrocentric chromosome are shown in Table 3. The data revealed an apparent decreasing trend in the amount of Ag stain from controls to cancer, except in mild dysplasia cases. The decrease in the amount of Ag stain per acrocentric chromosome in cancer and severe dysplasia cases was almost similar. The data from both mean NOR value and amount of Ag stain per chromosome indicated decreased activity of rDNA regions in both cancer and severe dysplasia cases, in comparison with controls and mild-to-moderate dysplasia cases.

DISCUSSION

Our results indicate a decrease in the frequency of Ag-NORs in cancer cases, in comparison with controls. The data also revealed an apparent decrease of Ag-NORs in severe dysplasia cases when compared with controls. The trend of decrease in the frequency of Ag-NORs in cancer and severe dysplasia cases is interesting, because the progression rate of this severe grade of precancerous lesion to cancer was reported to be significantly more in comparison with milder forms of precancerous groups [20–23].

The clinical significance of Ag-NOR activity is not known; however, there have been several reports on the decrease of Ag-NORs in leukemia and lymphoma [11–14]. A significant decrease of Ag-NORs was also reported in a case of CML in blastic crisis [24]. Excluding the report by Cheng et al. [15], no data are available thus far on the increase of Ag-NOR frequency in patients with adenocarcinoma. Recently,

Schulze et al. [14] found no detectable increase in the number of active NORs in cells of patients with malignant disease. However, a remarkably low NOR activity was observed in infectious mononucleosis. Evidence also showed that tumor cells release certain factors that had varied effects on circulating lymphocytes [18].

Some unknown serum factor may be responsible for suppressive action on rDNA transcription. However, the relation of rDNA activity and Ag-NOR is not clear, since inhibition of RNA synthesis by actinomycin D did not give consistent results [25].

Our analysis of the amount of Ag stain also revealed a decreased value per acrocentric chromosome in cancer (0.85) and severe dysplasia cases (0.84), in comparison with controls (1.05). It is likely that this variation in the amount of Ag stain is due to difference in the amount of rDNA, because hybridization in situ [26, 27] and filter hybridization [28] demonstrated that acrocentric chromosomes differ in the number of copies of rRNA genes they carry. In view of the nature of Ag stained material at the NOR [29], however, it is likely that this may not account for all the variations of Ag stain we have seen in our material.

In our analysis, the data did not show any age relationship with the frequency of Ag-NORs, as was reported by an earlier study on normal individuals [30]. The reasons for such difference in observation is not clear. Further comment on age relationship of Ag-NOR from this study may not be possible, since we do not have individuals of all age groups, especially patients younger than age 20.

In view of the variable biologic behavior of the dysplastic lesions of the uterine cervix, the observation of reduced activity of Ag-NOR in both cancer and severe dysplasia cases seems to be significant. Various prospective studies have demonstrated higher progression rates for severe dysplasia cases, compared with other milder groups of dysplasia. The decrease of Ag-NOR in severe dysplasia indicates the possibility that this phenomenon may have some implication in relation to the development of malignancy. The exact relation of the activity of ribosomal genes with malignancy is not known. The question regarding the expression of Ag-NORs and the development of malignancy appears to be of interest.

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